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# Limitations in converting waste gases to fuels and chemicals

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Carbon dioxide remediation is of vital importance in mitigating the impact of greenhouse gases on climate change. While various technologies have been presented in the literature, we argue that only by valorizing $CO_2$ capture can such technologies reach widespread adoption in the current geo-political disposition. One such option is $CO_2$ fixation by autotrophic bacteria into bio-diesel and commodity chemicals. While proof of concept technologies have been published in the literature, yet key limitations exist, including maximal yield of aerobic $CO_2$ fixation, and growth rates, productivities, and titers of anaerobic $CO_2$ fixation. Researchers are currently addressing these issues through metabolic engineering and the controlled supplementation of secondary metabolites.

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## Limitations in converting waste gases into fuels and chemicals

Fixation of carbon dioxide ($CO_2$) and methane ($CH_4$) serves a crucial role in the carbon cycles of Earth [1,2]. The past 100 years of industrialization destabilized this cycle, increasing the concentration of atmospheric greenhouse gases and raising concerns of climate change. Since $CO_2$ is the key driver of climate change [3,4], the focus of this review will be $CO_2$ fixation.

Humans release $CO_2$ in substantial quantities each year. The amount released and the atmospheric concentration continue to rise [2]. Many researchers are interested in capturing and sequestering these gases [5], yet there is little economic or political incentive to do so [6]. One might consider $CO_2$ fixation in current industrial processes, however these processes only consume 120 megatons as compared to the 9 gigatons released [7,2]. Another possibility is enhanced oil recovery. Operators are incentivized with higher well yields while the $CO_2$ is also stored underground, but one must consider the cost to transport the gas to the well site [8]. Increasing quantities of $CO_2$ are released by 2nd and 3rd world nations for whom there is little incentive for $CO_2$ capture or transportation to sequestration sites [2,9].

This review will focus on recent advances and technologies that valorize $CO_2$ fixation, their limitations, and efforts to overcome those limitations. We believe that technologies with an economic incentive are more likely to be implemented, particularly in 2nd and 3rd world nations, in comparison to technologies subsidized through legislation and taxes. Moreover product scales must be considered. If we wish to re-mediate gigatons of $CO_2$ yearly, the product market size must also be in the gigatons per year range [10].

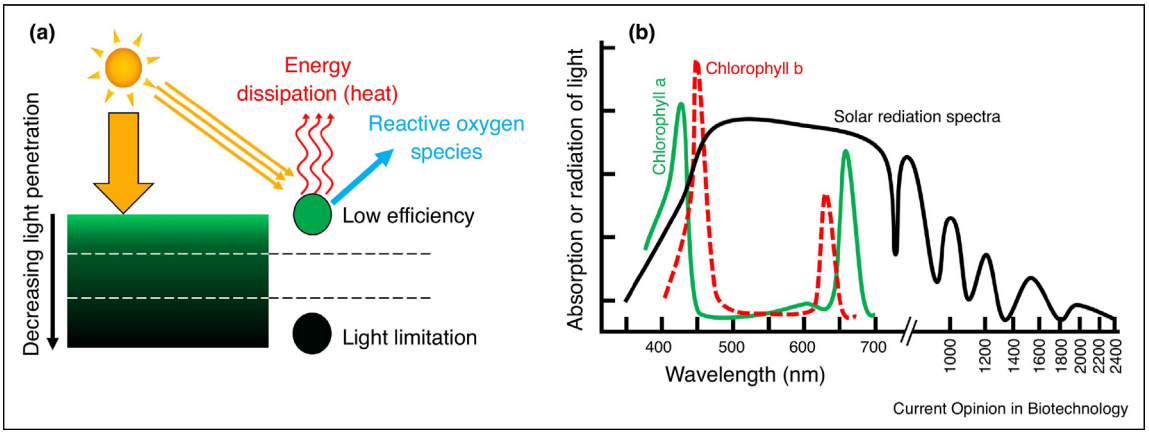
Only recently has the importance of gas fixation emerged as a generally applicable technology for the production of valuable chemicals from $CO_2$ [11]. Current biological gas fixation has key limitations, including slow growth rates and biomass density in defined media, low product titers [12••], and a limited range of products [13]. Researchers address these limitations through intelligent substrate selection, metabolic engineering, and bio-reactor process design. This review focuses on these limitations, and how they may be overcome.

## Improving photosynthetic efficiency and productivity

Photosynthesis is the predominant pathway for $CO_2$ fixation. Energy from the sun reduces $CO_2$ into sugars with RuBisCO and the Calvin-Benson (CB) cycle, while producing $O_2$. Though plant biomass production absorbs 3 gigatons of carbon [2] each year, there is little possibility for terrestrial carbon removal through plant growth because of land requirements, fertilizer costs, and the impact on food production [14]. Though there is interest in improving crop photosynthesis for the purpose of global food demand [15,16•], the current overall energy efficiency is around 1 to 7% [17,18].

In comparison to plants, algal systems have faster biomass productivities (by 30 times) [19] and can be cultivated in waste or brackish water [20]. Yet some photosynthetic limitations remain, including regions of sub-optimal light density within algal bio-reactors [21], very low algal biomass densities, and major portions of the suns spectrum that cannot be absorbed by chlorophyll (Figure 1). The former

**Figure 1**




Limitations for photosynthesis in algal photobioreactors. **(a)** Depth and algal concentration affect efficiency of photosynthesis and light starvation. **(b)** Chlorophyll absorb only certain wavelengths of light, some regions are under utilized. (a) Modified from [21]. (b) Generalized from [59].

can be solved with novel reactor design, and the latter can be engineered [16•] or isolated from nature [22].

## Aerobic $CO_2$ fixation with the Calvin-Benson cycle

Aerobic $CO_2$ fixation encompasses a broad set of pathways that power $CO_2$ fixation with ATP production from $O_2$ reduction. In contrast to photosynthesis, aerobic $CO_2$ fixation requires the consumption of $O_2$. Though rates and biomass densities of aerobic $CO_2$ fixation are sufficient, pathway efficiency limits the applications of these types of pathways.

Aerobic $CO_2$ fixation can be coupled with photovoltaics to create artificial photosynthesis, which could address the issues associated with plant and algal photosynthesis. This artificial photosynthetic process was recently demonstrated, and achieved an efficiency greater than that of natural photosynthetic systems during rapid growth phases (10% versus 5 to 7%) [23••]. In this process, *Ralstonia eutropha* fixated $CO_2$ to biomass and polyhydroxybutyrate (a bio-plastic). Despite the higher efficiency, titers remained in the mg $L^{-1}$ scale, with growth occurring over the course of 6 days. Higher productivities and titers will be necessary prior to implementation, for which there is precedence in the literature [24].

Much like plants and algae, which also operate the CB cycle for $CO_2$ fixation, chemolithoautotrophs like *R. eutropha* require substantial quantities of ATP to reduce $CO_2$ into biomass and other metabolites (Table 1), as compared to the Wood-Ljungdahl pathway (WLP).

**Table 1**

**$CO_2$ fixation pathways, and the ATP and $H_2$ cost to produce 1 mole of acetate, and the $H_2$ efficiency. Adapted from [60,61,18]. $H_2$ efficiency is calculated from the reducing equivalents in acetate (4 mol) divided by the total $H_2$ to fixate $CO_2$ and generate ATP. For aerobes, this $H_2$ is lost. (a) There is net production of ATP during $CO_2$ fixation, as high energy electrons on reduced ferredoxin are transferred to $NAD^+$, generating net proton motive force to drive ATP production [49]. (b) Unlike the other pathways, $H_2$ is not directly oxidized to generate ATP. (c) Assumes biomass is also a product. (d) The CB cycle is assumed to generate glyceraldehyde 3-phosphate (G3P) [62]; oxidative glycolysis converts this first to pyruvate and then acetyl-CoA by pyruvate dehydrogenase, losing 1 mole of $CO_2$ per acetyl-CoA. (e) Non-oxidative glycolysis is a theoretical bypass that converts all the carbon of G3P into acetyl-CoA and decreases the overall ATP cost per acetyl-CoA. (f) The enzymes required for non-oxidative glycolysis, in addition to oxidative glycolysis, are not included in this number as it is dependent on the organism [63]**

| | Requirements for the production of 1 Mol Acetate | | | | |
|---|---|---|---|---|---|
| Pathway | Mol Reducing equivalent | Mol ATP | Total Mol $H_2$ | Number of enzymes | $H_2$ efficiency |
| WLP | 2 NADPH + 2 $Fd_{red}$ | −0.63 [a] | 4.25 [b] | 8 | 100% [c] |
| 3HP/4HB cycle | 4 NADPH | 5 | 7 | 13 | 57% |
| Reductive TCA cycle | 4 NADPH | 1 | 5 | 8 | 80% |
| Calvin-Benson cycle (OxGly) | 4 NADPH | 6 [d] | 7.5 | 10 | 53% |
| Calvin-Benson cycle (NonOxGly) | 4 NADPH | 5 [e] | 7 | 10 [f] | 57% |

The 3HP/4HB cycle and reductive TCA cycle also require more ATP than the WLP. For aerobes, ATP is produced by $O_2$ respiration. While the CB cycle does require more ATP to fixat $CO_2$, $O_2$ respiration sufficiently provides this ATP resulting in industrially relevant titers and productivities.

The issue, instead, is the lower yield. Ultimately for aerobic $CO_2$ fixation, the $H_2$ used in $O_2$ respiration is lost, resulting in efficiencies less than 57%, and this has important implications on the economics when one wants to solve the $CO_2$ gigaton problem by producing large scale products such as diesel (Table 1).

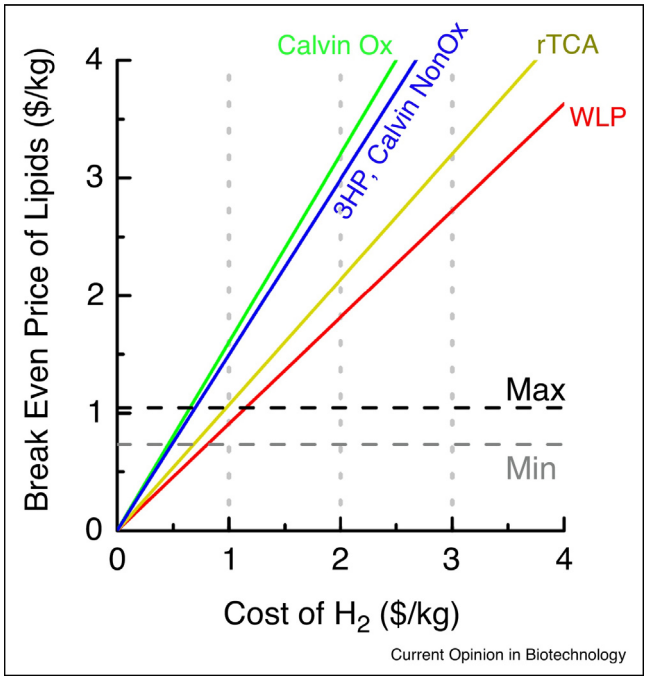
Take for instance $CO_2$ fixation and production of bio-diesel (such as lipids from yeast). First, $H_2$ is generated photolytically, and used to fixate $CO_2$ into acetate. Acetate can be fermented aerobically with *Yarrowia lipolytica* into bio-diesel at high yields (0.16 $g_{lipid}\ g_{acetate}^{-1}$) and productivities (0.8 g $L^{-1}$ $h^{-1}$) [12••,25•]. The cost of the $H_2$ directly determines the break even price of the bio-diesel based on the stoichiometric yield of each fixation pathway (Table 1). Say one purchases 1 kg of $H_2$ at 2 $/kg. If the CB cycle is used for $CO_2$ fixation and acetate production, and acetate is fermented into bio-diesel, at most only 0.64 kg of bio-diesel would be produced based on maximal pathway yields. The bio-diesel must be sold at 3.13 $/kg or 9.86 $/gallon to recoup the feedstock costs. As this is much higher than the current market price of diesel from oil and gas, the proposed bio-diesel process cannot compete (Figure 2).

Despite the simplification, which did not account for other processing costs, the discrepancy in these break even prices clearly indicates that the CB cycle and 3HP/4HB cycle require either very low $H_2$ costs [26], very high diesel prices, or substantial $CO_2$ fixation subsidies. Currently, it is unclear if any of these scenarios will occur. In this case, lipids or diesel would not be an ideal product. Instead, the organism should be engineered to produce a more valuable product that also has a large market size [27].

## Non-photosynthetic $CO_2$ fixation with the Wood-Ljungdahl pathway

Despite significant research over the past 76 years [28], only recently has the Wood-Ljungdahl pathway (WLP) risen to prominence for the ability to convert $H_2$ and $CO_2$ or CO into acetate and ethanol. This was in part due to the development of genetic tool kits for these acetogenic organisms [29], and an economic and political climate that valorizes the remediation of waste gases. Although the core biochemistry is yet to be fully resolved and fundamental research is ongoing concerning the regulation of the WLP [30], some mechanisms on transcriptional [31] and post-translational [32] control are already elucidated.

**Figure 2**

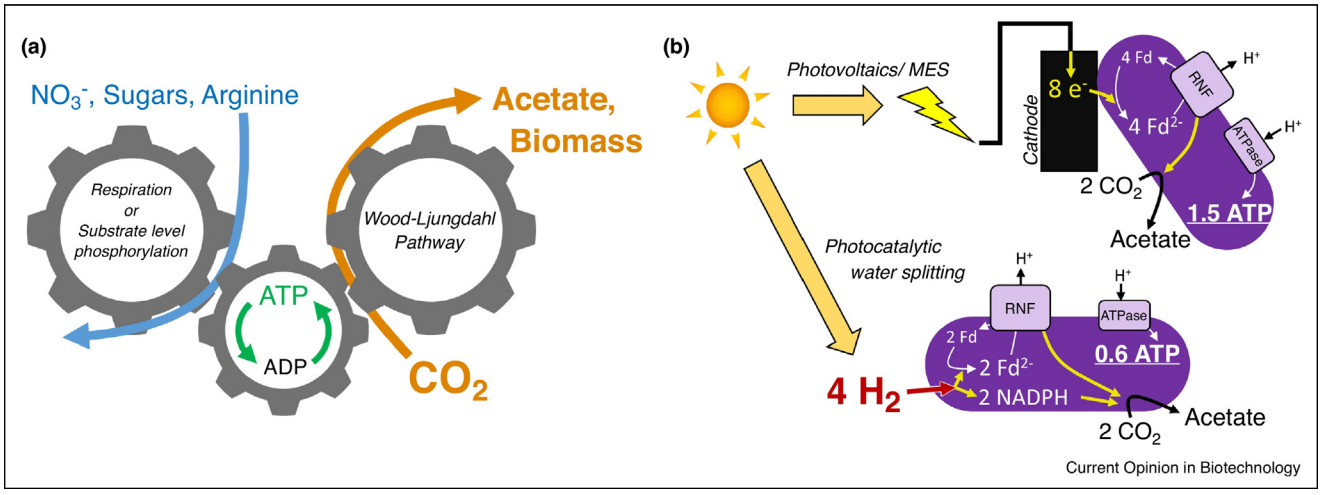



Break even price for lipids produced via $CO_2$ fixation by various pathways, and aerobic lipid accumulation, at given $H_2$ costs. This break even cost was based on the $H_2$ yield of each pathway to produce acetate (Table 1) and an experimental lipid yield of 0.16 $g_{lipid}\ g_{acetic\ acid}^{-1}$ by *Yarrowia lipolytica* [25•]. The minimum and maximum diesel price were plotted from between 2016 and May 2018 (EIA). Conversion to $ $gal^{-1}$ assumes a lipid/bio-diesel density of 0.832 kg $L^{-1}$.

The general principle of $CO_2$ fixation into a bio-diesel has already been demonstrated with an integrated bioprocess comprising an acetogen and a yeast [12••]. However, there were limitations to this process, including the utilization of 10 g $L^{-1}$ yeast extract. While the yeast extract was necessary to reach an acetate titer of 30 g $L^{-1}$, the cost of supplying this amount of yeast extract would be prohibitive for an economical process.

Despite the low ATP costs of $CO_2$ fixation (Table 1) acetogens generally grow slowly and to lower overall biomass densities and product titers. This is especially true when yeast extract (YE) is excluded from the media [33], though reactor design and media optimization can somewhat address these issues [34–36]. The potential cause for low biomass densities and product titers was well documented by Valgepea *et al.* [37••]. In brief, high biomass densities and acetate titers decrease the efficiency of ATP production, resulting in depletion of the acetyl-CoA pool, and collapse of metabolism. Researchers generally agree that anaerobic autotrophs are generally starved of ATP [38]. Providing an alternative source for the production of ATP could greatly impact growth rates, productivities, and titers (Figure 3).

**Figure 3**




Limitations for anaerobic $CO_2$ fixation. **(a)** Acetogens grown autotrophically are ATP deprived. ATP production can be supplemented with secondary metabolites such as nitrate or sugars. **(b)** Direct transfer of electrons to ferredoxin increases ATP yield per electron, as compared to splitting water, and feeding $H_2$.

### Respiration on other electron acceptors in tandem to $CO_2$ reduction

High aerobic rates are powered by the respiration of $O_2$ which powers ATP production with a highly negative $\Delta G^{0'}$ (Eq. (1)). Conversely, anaerobic autotrophic acetogens are ATP starved, because $CO_2$ reduction provides a smaller negative $\Delta G^{0'}$ for ATP synthesis (Eq. (4)).

A complementary source of ATP production could be utilized by acetogens, in tandem with $CO_2$ reduction. One such possibility would be respiration with an electron acceptor other than $O_2$ (Eqs. [(2) and [(3)) (Figure 3a). Nitrate, for example, has a $\Delta G$ close to that of $O_2$ [39], but seemingly prevents activity of the WLP in *Moorella thermoacetica* [40,41]. The mechanism for deactivation of the WLP in *M. thermoacetica* is currently unknown [42]. Yet recent research has shown that, while *M. thermoacetica* cannot respire on nitrate while reducing $CO_2$ with the WLP, *C. ljungdahlii* can. Nitrate improves growth rate, biomass density, and increases the ATP/ADP ratio and the acetyl-CoA pool size within the cell [43••].

The cost of nitric acid would be more expensive than sugars for the production of ATP, and would re-direct a percentage of the electrons from reducing $CO_2$. Conversely, nitric acid would also act as both nitrogen source and acid for pH balancing. The produced ammonium could also be harvested, then sold, or recycled to nitrate.

$$O_2 + 2H_2 \rightleftharpoons 2H_2O \Delta G^{0'} = -237\,\mathrm{kJ}\,mol_{H_2}^{-1} \quad (1)$$

$$NO_3^- + 4H_2 + 2H^+ \rightleftharpoons NH_4^+ + 3H_2O \quad \Delta G^{0'} = -150\,\mathrm{kJ}\,mol_{H_2}^{-1} \quad (2)$$

$$SO_4^{-2} + 3H_2 + 2H^+ \rightleftharpoons S + 4H_2O \quad \Delta G^{0'} = -41\,\mathrm{kJ}\,mol_{H_2}^{-1} \quad (3)$$

$$CO_2 + 4H_2 \rightleftharpoons Acetate + 2H_2O \quad \Delta G^{0'} = -24\,\mathrm{kJ}\,mol_{H_2}^{-1} \quad (4)$$

### Mixotrophy with arginine, sugars, and methanol

Other researchers have demonstrated similar increases in growth rates on CO rich gases, at least, with the addition of arginine which provides ATP through substrate level phosphorylation (SLP) [44•]. The greatest limitation of this discovery is the cost. Utilizing arginine for ATP synthesis would cost an order of magnitude greater than that of $H_2$ and $NO_3^-$ or $H_2$ based on theoretical yields (Table 2).

Sugars are one of the cheapest sources of ATP (Table 2). They have also been proposed to improve yields of acetogens through mixotrophy, where sugars and gases are consumed simultaneously [45,46••,47•]. Jones *et al.* [46••] and Maru *et al.* [47•] showed that adding $H_2$ to sugar fermentations decreased $CO_2$ evolution of acetogens during sugar fermentations. However, they did not show that

**Table 2**

**The cost to generate ATP from common WLP substrates. Prices determined from theoretical yields [49] and bulk lab scale prices from Sigma-Aldrich and Airgas. $CO_2$ was assumed to be free, though there would likely be associated costs to remove $O_2$. Similarly, $O_2$ concentrations require more expensive and purer $H_2$. The cost basis was 9.58 $ per mol arginine (Sigma, 10 kg); 0.86 $ per mole nitric acid (Sigma, 70% purity, 6× 2.5 L); 0.33 $ per mol $H_2$ (Airgas, 260 std $ft^3$, Ultrahigh purity); 1.72 $ per mol fructose (Sigma, 25 kg); 0.11 $ per mol methanol (Sigma, 200 L)**

| Substrate | Theoretical ATP produced | $/mol ATP |
|---|---|---|
| 1 Arginine | 1.00 | 9.6 |
| 4 $H_2$ + $HNO_3$ | 1.50 | 1.2 |
| 4 $H_2$ | 0.63 | 0.5 |
| 1 Fructose | 4.63 | 0.4 |
| 4 Methanol | 2.50 | 0.2 |

exogenous $CO_2$, could be fixated, that is from sources other than the sugar. Only when CO was provided as a syngas mixture ($CO:CO_2:H_2:N_2$, 55:10:20:15), did Jones *et al.* [46••] show substantial increased yields. It remains unclear whether exogenous $CO_2$ fixation occurred, or if the improved yields were from CO fixation into acetate, which actually generates $CO_2$ [28]. A major limitation of these articles was the focus on improving sugar fermentations with $H_2$ or CO, instead of improving $CO_2$ fixation with sugars.

### Electrosynthesis to bypass electron bifurcation of $H_2$

During growth on $H_2$+ $CO_2$, one limitation on ATP production is the bifurcation of electrons between reduced ferredoxin ($Fd^{-2}$) and NADPH [48]. Only $Fd^{-2}$ can drive proton motive force (PMF) with the RNF complex and ATP production [49], as the electrons of NAD(P)H are not at a high enough energy. For this reason, and because CO reduction only produces $Fd^{-2}$, ATP yields and growth rates are higher on CO than $H_2$ [13,33].

Take for instance the scheme proposed earlier where $H_2$ would be produced electrolytically, perhaps by solar power. If instead, the electricity was directly fed to acetogens, for the reduction of ferredoxin, ATP yields would approach that observed with CO (Figure 3b). Proof of concept microbial electrosynthesis (MES) has been developed for a variety of acetogens for the production of acetate and other organic compounds [50,51,52•]. Yet, there is currently no proof of electron transfer to ferredoxin in whole cells, though purified ferredoxin from *Clostridium pasteurianum* could be electrochemically reduced [53]. Alternatively, the electrons could be delivered to the RNF complex by another mechanism, though this remains speculative [54] and the effect of MES on the energetic state of the acetogenic cell has not been studied [50].

Current research has focused on how thermodynamics govern yields and efficiencies [55], and developing an understanding of how the cellular communities interact at the electrode [56•]. The mechanisms by which electrons are transferred remain poorly defined. This was reviewed in depth by Kumar *et al.* [57••]. Only after we understand these mechanisms can researchers develop metabolic engineering strategies to improve electron transfer efficiencies [58].

## Conclusion

$CO_2$ remediation is of paramount importance to minimize the negative impacts of climate change. Various technologies have been presented in the literature, however, we contend that only technologies that valorize the captured $CO_2$ have a realistic chance of widespread adoption. Furthermore, the product market size should be comparable to our $CO_2$ production, so as to have significant impact on climate change. One such option is the production of bio-diesel or chemicals with $CO_2$ fixating autotrophic bacteria. First, yields and prices dictate which pathways are viable for which products. For instance, the low yields of aerobic autotrophs make them unsuitable for bio-diesel production when the electron source is $H_2$ from photocatalytic water splitting. While the yields of anaerobic autotrophs are suitable, substantial work is necessary to improve growth rates, biomass density, and product titers. The general limiting factor is that anaerobic autotrophs are starved for ATP. This can be alleviated in a variety of ways, including supplementation with additional electron acceptors (nitrate), electron donors (sugars), or passing the electrons directly to ferredoxin (microbial electrosynthesis). We believe that these barriers can be overcome with current methods and technologies. If widespread adoption relies on valorizing $CO_2$ fixation, and the product target is the large scale by relatively inexpensive bio-diesel, the key limiting factor will become the cost at which reducing equivalents $H_2$ can be acquired.

## Author contributions

D.F.E. and G.S. wrote the manuscript. All authors read and approved the final manuscript.

## Conflict of interest statement

Nothing declared.

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